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Artificial Oocyte Activation with Calcium Ionophore May Improve Implantation of Potential in Recalcitrant Infertility

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ABSTRACT

This case report describes a couple who achieved two live births after seven failed in vitro fertilization cycles by the addition of artificial oocyte activation (AOA) to the intracytoplasmic sperm injection (ICSI) used in their normal course of treatment. Their fertilization history was within normal limits, but implantation failed to occur after several fresh embryo transfers as well as preimplantation genetic tested (PGT) frozen embryo transfers. The addition of AOA may have augmented the patient's typical oocyte activation, leading to more robust embryo development and thus implantation. The safety and efficacy of the technique is reasonably established and should be considered an option before recommending donor gametes to patients in cases of repeated implantation failures.

Key words: Artificial oocyte activation, Calcium ionophore, Refractory infertility, Intracytoplasmic sperm injection, In vitro fertilization-embryo transfer.

INTRODUCTION

Successful human oocyte fertilization and embryogenesis requires initial oocyte activation. Both the sperm and the oocyte contribute activating factors. These drive fertilization events by stimulating calcium ion (Ca++) release from the endoplasmic reticulum into the cytoplasm in waves, or oscillations. Cortical granule release, resumption and completion of meiosis, and pronuclei formation all rely on these oscillations, and without them, pregnancy cannot occur [1-3].

Natural fertilization in vivo begins with fusion of the sperm and oocyte membranes. Phospholipase C zeta (PLC-z) diffuses out of the sperm from the post-acrosomal region and hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP2) into inositol-1,4,5-triphosphate (IP3), a vital activation molecule. IP3 receptors are distributed throughout the oocyte cortex as well as on membranes of smooth endoplasmic reticulum (sER) and mitochondria. Once IP3 binds to the target areas, Ca++ is released from stores in the sER, initiating the first steps in fertilization [4,5].

The first peak of cytoplasmic Ca++ occurs around 20-40 minutes after sperm penetration [6]. It lasts about 4 minutes, activating protein kinase C molecules (PKCs). These PKCs translocate to the oocyte cortex and disrupt the actin cytoskeleton, causing the contents of the cortical granules to exocytose outward, toward the zona pellucida [2]. They remodel the zona pellucida's glycoprotein matrix by cross-linking to block additional sperm penetration. This "polyspermy blocking" mechanism occurs rapidly and is the first noticeable event triggered by the Ca++ wave. [7] Each subsequent Ca++ oscillation has a decreasing amplitude; waves persist for an average of 4.2 hours and drive two other primary fertilization events, namely resumption of meiosis and formation of the two pronuclei of fertilization [8].

Prior to sperm penetration, the oocyte was arrested at the metaphase II (MII) stage of meiosis by cytostatic factor, or CSF, which inhibits anaphase-promoting complex (APC). Intracellular Ca++ spiking activates a set of reactions driven by calmodulin kinase II, which degrades CSF and eventually relieves APC inhibition and promotes completion of meiosis [6]. Concomitantly, sperm decompensation processes unwind the sperm chromosomes, which recombine with the oocyte's chromosomal complement, extrude a second polar body, and produce the visible fertilization pronuclei.

Energy requirements in the zygote skyrocket temporarily. Ca++ oscillations stimulate mitochondrial respiration to produce the adenosine triphosphate (ATP) necessary to power further calcium waves and subsequent normalizations (troughs between the peaks). Any calcium ion deficiencies must be replenished by pumping Ca++ into the cell from the environment. ATPases power these pumps, and maintain these opposite oscillatory responses. Insufficient Ca++ release means there will not be enough ATP produced to drive all the necessary processes [8-11].

Deficiencies in Ca++ oscillations may arise from the sperm or the oocyte. A sperm may deliver insufficient PLC-z, which results in too little PIP2 or IP3, thus dampening or failing to trigger Ca++ oscillations. The oocyte may contain an inadequate pool of Ca++ for release, or depleted mitochondria which cannot provide energy to maintain subsequent oscillations [9]. Whichever the cause, any significant deviation in Ca++ oscillation patterns results in poor or no fertilization and / or embryo development [12, 13].

In vitro fertilization and intracytoplasmic sperm injection (IVF-ICSI), while standard assisted reproductive techniques, are vulnerable to these same gamete deficiencies. ICSI bypasses typical sperm-oocyte membrane interaction and deposits a sperm directly into the cytoplasm, overcoming many if not most sperm anomalies, including oligoasthenoteratozoospermia, globozoospermia, low hypo-osmotic swelling scores, and antisperm antibodies [14-22]. Despite this mechanical assistance, between 1-5% of IVF-ICSI cases still result in total fertilization failure (TFF) despite adequate numbers of oocytes.

Fertilization failure despite ICSI can be related to a partial or complete inability of the sperm to activate the oocyte due to deficient or dysfunctional PLC-z, as noted. Male chromosome mutations which produce dysfunctional PLC-z may be a factor. PLC-z concentration is positively correlated with the frequency and amplitude of Ca++ spikes induced, so dysfunctional PLC-z results in lack of oocyte activation [4]. This defect is particularly marked in males with globozoospermia, where the sperm acrosome is missing, and potentially some of the post-acrosomal region as well [15,16,21]. ICSI performed with these sperm produces a low fertilization range: 0-37% [14-21].

One manipulation technique to reduce the risk of TFF is sperm immobilization prior to injection. The sperm membrane is destabilized during mechanical immobilization by the ICSI micropipette, rendering sperm more

controllable during injection and possibly allowing more efficient PLC-z diffusion once deposited in the cytoplasm [12]. Electrical activation of oocytes has successfully obtained fertilization but is not often utilized. Several chemical activators, such as strontium chloride or ionophores such as ionomycin and calcimycin, can also be used [15,22,24,25].
These chemical methods, specifically calcimycin or "calcium ionophore" (A23187) is the most common means of artificial oocyte activation, or AOA. Early efforts, near the beginning of clinical ICSI use, showed that ICSI oocytes which failed to show fertilization 24 hours after injection could be stimulated to the 2-pronuclear fertilization stage by exposure to calcium ionophore [4]. This underlined the importance of Ca++ in the cascade of early fertilization events. A range of AOA protocols have been published. Some expose injected oocytes to AOA solution immediately after ICSI, others delay for 60 minutes post-injection; the length of exposure varies from 5 minutes to 30 minutes [24]. We selected and refined a protocol to closely mimic the natural Ca++ rise and involved a reasonably short exposure time, for safety [22]. Multiple meta-analyses now show that judicious usage of AOA after IVF-ICSI results in improved fertilization, cleavage, blastulation rate, and pregnancy rates in patients with a history of failed fertilization, suboptimal fertilization or embryo development, without increased risk of miscarriage or congenital birth defects [22-25].
Presented here is a case where treatment with ICSI and Ca++ ionophore resulted in a total of 2 live deliveries of babies after a long history of IVF failure elsewhere.

CASE REPORT

The couple had pursued treatment for 3 years. The female partner was 37 years old at the start of IVF attempts. She had rheumatoid arthritis, for which she took prednisone and adalimumab. The male partner had oligoasthenoteratospermia, with a sperm count of under 10 million, 25% motile, and 0% normal forms by strict criteria. They underwent a total of 7 stimulated IVF cycles using ICSI. Of these 7 cycles, 4 used ejaculated sperm, one fresh TESE sample (testicular sperm extraction) on a fifth, and 2 used frozen TESE sperm that was cryopreserved after the fresh TESE collection. Each cycle had a reasonable fertilization rate, ranging from 30-64%.
Three early IVF cycles utilized preimplantation genetic testing (PGT) of embryos as part of the couple's treatment plan. PGT results, respectively, were 1 low mosaic, then 2 aneuploid, then 1 euploid blastocyst. The euploid blastocyst was transferred into the patient on a frozen transfer cycle; she was pregnant for a few weeks but miscarried. The analyzed products of conception confirmed that the embryo had been euploid.
The couple opted for fresh embryo transfers and no PGT on the 4 subsequent IVF cycles. Each cycle had 1 blastocyst transferred, with no success. There were 2 additional frozen embryo transfers (FETs) of supernumerary embryos cryopreserved from these 4 IVF attempts, also resulting in no pregnancy.
When the couple presented at our infertility center, the female partner was now 39 years old. The treatment plan was IVF-ICSI with Ca++ ionophore to try to improve embryo development and increase chances of implantation.
Ovulation induction for their first cycle was an antagonist cycle (cetrorelix) and gonadotropins. The patient responded well to the medications. Final oocyte maturation trigger was 2 doses of leuprolide acetate given 12 hours apart, 34 hours prior to oocyte retrieval.
Twenty-four oocytes were collected. ICSI was performed on 23 MII oocytes approximately 4 hours post-collection. A timer was set for 30 minutes, at which time the injected oocytes were immersed in a 10 uM/L calcium ionophore

solution, specifically A23187 (calcimycin) for 7 minutes. They were then rinsed thoroughly and placed in fresh culture drops. Out of the 23, 15 showed 2 pronuclei the following morning.

On day 3, 12 were still developing normally. Three good quality 8 cell embryos were placed in the patient's uterus using ultrasound guidance after assisted hatching was performed on them via laser. Luteal phase support was provided. Eight 3-day old embryos of good quality were cryopreserved. The patient delivered a singleton full-term baby girl.

The couple returned a little over a year post-delivery, hoping for another child. Since there were 8 embryos cryopreserved, the couple underwent 2 frozen transfer cycles without success, depleting the stored embryos from the first IVF cycle.

The female partner, now 41 years old, underwent a second ovulation induction cycle using the same medications as above, except the trigger was human chorionic gonadotropin (hCG). Six oocytes were collected; 2 oocytes fertilized with ICSI with Ca++ ionophore treatment out of 5. Both were transferred into the patient on day 3 without successful pregnancy.

On the third IVF ovulation induction cycle, we followed the same medication protocol as on the second cycle. The female patient was still 41 years old. Seven oocytes were retrieved, six underwent ICSI with Ca++ ionophore. A total of 4 fertilized and 3 developed appropriately. All 3 were transferred on day 3 of culture: 2 at 8 cells, 1 at 6 cells. A singleton pregnancy was achieved. Despite intrauterine growth restriction (IUGR) diagnosis, the patient delivered a healthy girl.

The patient had rheumatoid arthritis. We have found that not only can dopaminergic drugs e.g., dextroamphetamine sulfate may markedly ameliorate the pain from rheumatoid arthritis and prevent further progression and damage by releasing more dopamine from sympathetic nerve fibers thus decreasing increased cellular permeability (which had allowed an excessive amount of irritants infiltrating joints and pelvic tissues. Dopaminergic drugs can also ameliorate many other chronic, possibly autoimmune conditions, and at the same time improve fecundity [26-35]. Thus, she was treated also with amphetamine salts 30mg twice daily providing 18.8 mg dextroamphetamine twice daily.

DISCUSSION

The simple addition of Ca++ ionophore treatment to this couple's IVF-ICSI cycles seems to have given just enough extra energetic potential to the embryos to achieve pregnancy and live delivery, despite the multiple previous failed cycles.

The female partner was age 39 when presenting at our infertility center initially, and by the 3rd cycle she was 41 years old, an age when embryo viability is decreasing rapidly. Oocytes from females of advanced age have a higher incidence of meiotic errors, possibly arising from decreased ATP to power chromosomal segregation and recombination. It is theoretically possible that insufficient oocyte activation could result in lower mitochondrial respiration and energy availability. One study suggested that ICSI-AOA in older patients produced higher cleavage

rates and significantly more top-quality embryos than ICSI alone. [36] This supports the possibility that use of calcimycin for AOA may help create embryos with improved implantation potential. Chen et al applied ICSI-AOA to patients having previous poor embryo development [10]. While all age groups benefited from use of Ca++ ionophore in their study, clinical and ongoing pregnancy rate were significantly higher in only the younger age group (<35 years [10].

The male partner, with a sperm normal morphology of 0%, could perhaps have had deficient or dysfunctional PLC-z in his sperm [12]. The 7 IVF cycles performed elsewhere without calcium ionophore treatment resulted in a normal fertilization rate, indicating that there was at least some PLC-z present in the sperm.

In their first IVF cycle at our center, however, we noted that 3 of 15 embryos had arrested by day 3, suggesting that even with AOA this couple's gametes may occasionally have inadequate oocyte activation. Several studies have reported decreased embryo cleavage rates associated with inadequate Ca++ oscillations during fertilization steps. Murugesu et al found a pronounced decrease in cleavage rates in their meta-analysis, 68.3% in the AOA group vs 48.7% in the non-AOA group [37]. In the same analysis, blastocyst formation was also blunted: 51% in the AOA group vs 10.7% in the non-AOA group [37]. Shafqat et al mentions that the inner cell mass of blastocysts produced from Ca++-devoid fertilization is observably smaller [3]. These observations suggest that inadequate oocyte activation may show more pronounced effects in later stages of embryo development.

CONCLUSION

This case report demonstrates that ICSI-AOA can provide more robust treatment for patients who have had multiple failed IVF cycles using their own gametes. Adding calcium ionophore activation can provide a successful outcome. Furthermore, the safety of calcium ionophore use has been reasonably established and is not detrimental [25]. The addition of AOA to the treatment regimen may have allowed the achievement of a live healthy baby with the female partner's own egg rather than proceeding to the use of donor eggs which was suggested to her by 2 different IVF centers.

LIMITATIONS

1. Though the couple had their IVF-ET cycles at well-respected IVF centers with good, published success rates, it is possible that some nuances in our IVF or ET methodology of controlled ovarian hyperstimulation or our type of controlled ovarian hyperstimulation could be responsible for her 2 successful pregnancies rather than AOA.

2. Similarly, the successes at our infertility center could have been more related to the use of dopaminergic drugs to negate to some degree the potential of excessive cellular immune activity with the rejection of the fetal semi-allograft. It should be recalled that she did have a miscarriage following one of the IVF cycles with a fetus with normal chromosomes and continual P support [29].

3. If indeed AOA played a significant role on both of the successful outcomes, it may be that AOA despite fertilization and blastulation only helps a minority of cases with repeated IVF failures despite multiple transfers of embryos It would be difficult to attain a large enough series of similar patients because most couples would not have gone through so many IVF cycles with failures and still keep trying. Certainly, a randomized controlled trial would not even be a consideration. This case will encourage our group to try it on some similar cases and perhaps if we have tried it in a small series, we will try to share our data through publication of another manuscript whether the outcome is supportive of AOA benefits or not. It is also hoped that this report could influence another IVF center to try it on similar cases and hopefully also find a beneficial effect of AOA.

REFERENCES

1. Mann JS, Lowther KM, Mehlmann LM. Reorganization of the Endoplasmic reticulum and development of Ca2+ release mechanisms during meiotic maturation of human oocytes. Biol Reprod. 2010; 83(4): 578-583.
2. Ducibella T, Fissore R. The roles of Ca2+, downstream protein kinases, and oscillatory signaling in regulating fertilization and the activation of development. Dev Biol. 2008; 315(2): 257-279.
3. Shafqat A, Kashir J, Alsalameh S, Khaled Alkattan K, Yaqinuddin A. Fertilization, oocyte activation, calcium release and epigenetic remodelling: lessons from cancer models. Front Cell Dev Biol. 2022; 10:781953.
4. Tesarik J, Mendoza C, Greco E. The activity (calcium oscillator?) responsible for human oocyte activation after injection with round spermatids is associated with spermatid nuclei. Fertil Steril. 2000; 74(6):1245-1247.
5. Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. Nature. 1996; 379(6563): 364-368.
6. Dozortsev D, Qian C, Ermilov A, Rybouchkin A, DeSutter P, Dhont M. Sperm-associated oocyte-activating factor is released from the spermatozoon within 30 minutes after injection as a result of the sperm oocyte interaction. Hum Reprod. 1997; 12(12):2792-2796.
7. Rojas J, Hinostroza F, Vergara S, Pinto-Borguero I, Aguilera F, Fuentas R, Carvacho I. Knockin' on egg's door; maternal control of egg activation that influences cortical granule exocytosis in animal species. Front Cell Dev Biol, 2021; 9:704867.
8. Sanders JR, Swann K. Molecular triggers of egg activation at fertilization in mammals. Reproduction. 2016; 152(2): R41-R50.
9. Anifandis G, Messini CI, Dafopoulos K, Daponte A, Messinis IE. Sperm contributions to oocyte activation: more than meets the eye. J Assist Reprod Genet. 2016; 33(3):313-316.
10. Yeste M, Jones C, Amdani SN, Patel S, Coward K. Oocyte activation deficiency: a role for an oocyte contribution? Hum Reprod Update. 2016; 22(1):23-47.
11. Reynier P, May-Panloup P, Chretien MF, Morgan CJ, Jean M, Savagner F, Barriere P, Malthiery Y. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod. 2001; 7(5):425-429.

12. Yuan P, Yang C, Ren Y, Yan J, Nie Y, Yan L, Qiao J. A novel homozygous mutation of phospholipase C zeta leading to defective human oocyte activation and fertilization failure. Hum Reprod. 2020; 35(4):977-985.
13. Mu J, Zhang Z, Wu L, Fu J, Chen B, Yan Z, Li B, Zhou Z, Wang W, Zhao L, Dong J, Kuang Y, Sun X, We L, Wang L, Sang Q. The identification of novel mutations in PLCZ1 responsible for human fertilization failure and a therapeutic intervention by artificial oocyte activation. Mol Hum Reprod. 2020; 26(2): 80-87.
14. Van Steirteghen AC, Nagy Z, Joris H, Liu F, Liu HJ, Staessen C, Smitz J, Wisanto A, Devroey P. High fertilization and implantation rates after intracytoplasmic sperm injection. Hum Reprod. 1993; 8(7):1061
15. Rybouchkin AV, Van der Straeten F, Quatacker J, DeSutter P, Dhont M. Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round headed sperm associated with deficient oocyte activation capacity. Fertil Steril. 1997; 68(6):1144-1147.
16. Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round headed sperm. Fertil Steril. 1997; 68(1):118-122.
17. Check ML, Check JH, Summers-Chase D, Choe JK Check DJ, Nazari A. Live birth after posthumous testicular sperm aspiration and intracytoplasmic sperm injection with cryopreserved sperm: Case report. Clin Exp Obstet Gynecol. 2002; 29(2):95-96.
18. Check JH, Katsoff D, Check ML, Choe JK, Swenson K. In vitro fertilization with intracytoplasmic sperm injection is an effective therapy for male factor related to subnormal hypo-osmotic swelling test scores. J Androl. 2001; 22(2):261-265.
19. Check JH, Hourani W, Check ML, Graziano V, Levin E. Effect of treating antibody-coated sperm with chymotrypsin on pregnancy rates following IUI as compared to outcome of IVF/ICSI. Arch Androl. 2004; 50(2):93-95.
20. Check ML, Check JH, Katsoff D, Summers-Chase D: ICSI as an effective therapy for male factor with antisperm antibodies (ASA). Arch Androl. 2000; 45(3):125-130.
21. Liu J, Nagy Z, Joris H, Tournay H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. Hum Reprod. 1995; 10(3):626-629.
22. Pinto J, Check JH. Correction of failed fertilization despite intracytoplasmic sperm injection with oligoastheno-terato zoospermia but with acrosomes present by oocyte activation with calcium ionophore – case report. Clin Exp Obstet Gynecol. 2008; 35(4):252-254.
23. Crafa A, Condorelli RA, La Vignera S, Calogero AE, Cannarella R. Globozoospermia: a case report and systematic review of literature. World J Mens Health. 2023; 41(1):49-80.
24. Check JH, Summers-Chase D, Cohen R, Brasile D. Artificial oocyte activation with calcium ionophore allowed fertilization and pregnancy in a couple with long term unexplained infertility where the female partner had diminished egg reserve and failure to fertilize oocytes despite intracytoplasmic sperm injection. Clin Exp Obstet Gynecol. 2010; 37(4):263-265.

25. Check JH, Summers D, Horwath D, Sobel M, Neumann B. A review of artificial oocyte activation with calcium ionophore for fertilization failure and a case report of a successful twin pregnancy. Gynecol Reprod Health. 2023; 7(4):1-6.
26. Boimel P, Check JH. Marked improvement of intractable arthritic pain in a woman with rheumatoid arthritis with sympathomimetic amine treatment despite previous failure with standard therapy and possible implications for last trimester unexplained fetal demise – case report. Clin Exp Obstet Gynecol. 2007; 34(4):254-256.
27. Check JH. Most chronic conditions in women are related to increased cellular permeability and most can be effectively treated with dopaminergic drugs. J Biomed Res Environ Sci. 2024; 5(4):373-386.
28. Check JH. Treating pelvic pain and endometriosis with the goal of correcting infertility and/or preventing miscarriage. J Reprod Med Gynecol Obstet. 2024; 9:167.
29. Check DL, Check JH. Novel methods of improving fecundity and various pathological disorders based on a hypothetical model of embryo implantation. Gynecol Reprod Health. 2020; 4(4):1-15.
30. Check JH, Chern R, Katsoff B. Prevention of first-trimester miscarriage with dextroamphetamine sulfate treatment in women with recurrent miscarriage following embryo transfer – case report. Clin Exp Obstet Gynecol. 2014; 40(4):471-472.
31. Check JH, Check DL, Neumann B. A Novel Treatment of Crohn’s Disease that is Not Only Safe in Pregnancy but Can Correct Infertility and Recurrent Miscarriages. Gynecol Reprod Health. 2024; 8(2):1-9.
32. Check JH, Neumann B. Combined Dopaminergic Drugs with Supplemental Progesterone to Treat Recurrent Miscarriage and Ulcerative Colitis despite Diminished Egg Reserve. Gynecol Reprod Health. 2024; 8(2):1-10.
33. Check JH, Neumann BA. Correction of treatment refractory infertility and severe constipation following treatment with supplemental progesterone and a dopaminergic drug. Gyn Reprod Health. 2024; 8(3):1-9.
34. Check DL, Check JH. Novel methods of improving fecundity and various pathological disorders based on a hypothetical model of embryo implantation. Gynecol Reprod Health. 2020; 4(4):1-15.
35. Check JH, Aly J, Chang E. Improving the chance of successful implantation – part I – embryo attachment to the endometrium and adequate trophoblast invasion. Clin Exp Obstet Gynecol. 2016; 43(6):787-791.
36. Check JH, Aly J. Improving the chance of successful implantation – part 2 – Circumventing immune rejection and the fetal semi-allograft. Clin Exp Obstet Gynecol. 2018; 45(1):9-13.
37. Tsai TE, Lin PH, Lian PF, Li CJ, Vitale SG, Mikus M, Su WP, Tsai HW, Tsui KH, Lin LT. Artificial oocyte activation may improve embryo quality in older patients with diminished ovarian reserve undergoing IVF-ICSI cycles. J Ovarian Res. 2022; 15(1):102-110
38. Murugesu S, Saso S, Jones BP, Bracewell-Mines T, Athanasiou T, Mania A, Serhal P, Ben-Nagi J. Does the use of calcium ionophore during artificial oocyte activation demonstrate an effect on pregnancy rate? A meta-analysis. Fertil Steril. 2017; 108(3):468-482.e3